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AN AQUEOUS SOLUTION OF HEMATOXYLIN WHICH DOES NOT READILY DETERIORATE.

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For most of the purposes of histology there is a no more satisfactory and generally applicable stain than hematoxylin, and experience has shown that aqueous solutions are on the whole preferable to those containing a considerable quantity of alcohol. Every microscopist knows, however, that aqueous solutions of hematoxylin soon begin to deposit a dark precipitate on the bottle and become filled with granules and frequently with threads.

As so many changes are due to living ferments (organized ferments, as bacteria, yeast-like fungi, etc.), it occurred to the writer that the deterioration of the hematoxylin might be due, wholly or in part, to one or more of these organized ferments. If this supposition is correct, any ferments accidentally present in the water and alum used, should be destroyed. If then some substance is added to the solution to prevent the development of germs that might subsequently get into the solution, the solution would be likely to be lasting and to retain its excellence. Experiments proved the accuracy of the hypothesis. An aqueous solution of hematoxylin prepared as directed below and made nearly a year previous to the present writing is still in excellent condition. During the year it has been in the laboratory and subjected to all the vicissitudes of heat, dust, light, and all the other conditions that an ordinary histological re-agent must endure. It has remained in the same bottle all the time, and now, after nearly a year, there is no deposit on the bottle, and the stain acts as well as in the beginning.

Formula :

Distilled water..........................	200 cc.
Potash or ammonia alum................	7½ grams.
Chloral hydrate.........................	4 grams.
Hematoxylin crystals	$\frac{1}{10}$ gram.

To prepare the solution, place the water and alum in an agate or porcelain vessel and boil for five minutes or more, preferably fifteen or twenty minutes if the water is not freshly distilled. This boiling will destroy any germs in the water or alum. Allow the boiled alum solution to cool, and add sufficient freshly boiled distilled water to compensate for evaporation and make 200 cc. of alum water. Add the chloral hydrate and dissolve the hematoxylin crystals in 10 cc. of 95 per cent. alcohol and add to the mixture.

At first the color will be rather faint, but after a week or two it will become a deep purple. The deepening of the color is more rapid if the bottle is left uncorked in the light and is shaken occasionally.

If the stain is too concentrated it may be diluted with freshly distilled water or with a mixture of water, alum, and chloral. If the stain is not sufficiently concentrated more hematoxylin may be added. With hematoxylin of the strength given in the formula, sections are usually sufficiently stained in from one to five minutes.

As may be inferred from what was said above, the boiling is to destroy any living ferments present in the water or alum, and the chloral hydrate is to prevent the development of germs which accidentally reach the solution after it is made.

No precaution is necessary in using this stain for sections, except that applicable to all hematoxylin solutions, viz: after staining, the surplus stain must be very thoroughly washed away with distilled water; otherwise black granules or needles will appear in or upon the sections. If granules appear in the preparations in spite of the washing, it will be well to boil the solution three to five minutes and filter through paper or absorbent cotton. The addition of one or two per cent. of chloral after the boiling is also advantageous. This stain has not been tried for dyeing in bulk. Other substances than chloral were tried, but not with so good success. For stains other than hematoxylin, chloral might not prove so satisfactory a preservative.

As hematoxylin is, for most tissues, almost a pure nuclear dye, it has been found advantageous to stain the cell body, ground substance, etc., with a general or counter stain. One of the most satisfactory of these is eosin, prepared as follows:

Distilled water	50 cc.
95 per cent. alcohol	50 cc.
Dry eosin	$\frac{1}{10}$ gram.

The eosin is used after the hematoxylin in most cases, and, as it is in alcoholic solution, it may be washed off with 95 per cent. alcohol if the object is to be mounted in balsam. If it is to be mounted in glycerin or glycerin jelly, the excess of eosin should be washed away with distilled water.

An excellent counter-stain for many objects is picric acid. One proceeds with this as with the eosin, except that a saturated alcoholic solution is made and after the hematoxylin is washed away a drop of the picric acid solution is added. This is then washed away with 95 per cent. alcohol. The time required for the eosin or the picric acid to stain is very short, usually only a few seconds.*

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* For previously described solutions of hematoxylin, see Lee, The Microtomist's Vade Mecum, 2d ed.